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The work initiated under this contract will be continued as part of a program of which Dr. W. Wegendank is the principal investigator.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The electrical potential at the boundary of the outside and inside of biological cell phases is studied on the basis of the Association-Induction Hypothesis. According to the latter the cell interior contains fixed sites, in the side chains and at the backbones of proteins at which biologically active substances, like ions are specifically adsorbed. Equations have been derived to determine the profile of the electrical poten-
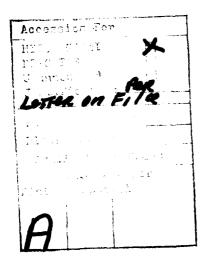
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tial at the phase boundary of the cell. They are to be solved when solutions to the wave mechanical problems described below will have been obtained. As the protein sites are interconnected cooperative mechanisms are involved which are due to inductive interactions. The basis of these interactions is wave mechanical. A method is used to determine the changes in the charge densities of the localized electrons as well as the conformational changes in proteins.

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THE ASSOCIATION-INDUCTION HYPOTHESIS AND PHYSIOLOGICAL EXCITATION

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Objective

The purpose of this research is to study physiological excitation on the basis of the Association-Induction Hypothesis.[1] One aim of this investigation is to derive theoretically the width of the electrical potential at the boundary of the inside and outside phases of a biological cell. Another aim is the theoretical derivation of the reversal of that potential during excitation.

Introduction

Physiological excitation leads from a stimulus, such as an applied electrical voltage or current, to the production of an action potential, if the stimulus strength is above a threshold value. It has been experimentally established[2] that excitability of living cells depends on the presence of sodium ions in the bathing medium. In addition, it has been shown[3] that the magnitude of the action potential depends on the external sodium concentration. The action potential, which has opposite polarity of the resting membrane potential, is initiated by an influx of sodium ions into biological cells, followed by an efflux of potassium ions[4]. Hence, there is a switch in the preference of cells from potassium ions in rest to sodium ions in excitation. This is explained on the basis of the Association-Induction Hypothesis by a change from specific adsorption of potassium ions to protein sides in resting cells to specific adsorption of sodium ions to

the same protein sites in excitation. It has been shown[5] that the changes occuring in the adsorbed potassium ions, in exchange for calcium ions, due to a cationic stimulus, give the right order of magnitude of the stimulus threshold and explain experimental subthreshold responses. Derivation[6] of the space profile of the electrical phase boundary potential at the cell surface under certain simplifications (see below) has shown that the effective width of that potential of the same order as that of the cell membrane determined experimentally. This had led to the interesting question whether the functional membrane is an electrical phase boundary layer instead of the structured anatomical cell membrane.

Another fundamental aspect of the Association-Induction Hypothesis is the involvement of cooperative interactions in the specific adsorption of ions and other biological substances at protein sites. As is well known from physics, the essential feature of cooperative phenomena is (nearest) neighbor interaction. On the basis of statistical mechanics a cooperative specific adsorption isotherm has been derived[7,8] which agrees with many experimental data[9-11]. These cooperative interactions have also been studied stochastically in good agreement with the above mentioned cooperative specific adsorption isotherm[12] as well as with experimental results[13].

The involvement of cooperative phenomena in physiological excitation has been found experimentally[14] and studied theoretically[15,16]. Another theoretical investigation[17], on the basis of cooperative specific adsorption, takes into account the changes in specificities[18] if protein sites for potassium or sodium depending on the desorption of the cardinal adsorbent[1] calcium, adsorbed at a cardinal site[1], from such a site due to a cathode as stimulus.

Also, water molecules can be specifically adsorbed at protein-sites and

backbones[19]. This leads t ensembles of cooperatively interacting water molecules which from polarized multilayers[19]. According to the Association-Induction Hypothesis this polarized water in cells excludes sodium ions from the intra-cellular space[19]. Hence, the Association-Induction Hypothesis leads to the concept that the inside of a cell has more the characteristics of a solid (or semi-solid) state rather than those of a free solution, as already concluded long ago[20].

Description of Methods and Results
Derivation of the Spatial Profile of the Electrical Phase Boundary

Potential

The above-mentioned theoretical investigaton[6] of the profile of the electrical phase boundary was based on the study of the dependence of the electrical potential in the outside and inside of a system consisting of two phases which correspond to the extracellular and intracellular spaces of a biological cell. The extracellular space was assumed to contain only the cations potassium and sodium at their physiological concentrations as well as an anion, for instance chloride, of which the concentration was the same as the sum of the potassium and sodium concentrations. It was assumed that the phase which corresponds to the inside of the biological cell contained a fixed anion of appropriate concentration. For reasons of simplification, the following assumptions were made: 1) the fixed anion is homogenously distributed throughout the inside, 2) the fixed anions are not interconnected as they are in protein, 3) the penetration of the mobile anion in the outside phase is neglected, 4) the boundary was considered so thin that there is no surface charge on it. The spatial profile of the electrical potential is determined[6] by the Poisson equation according to which the divergence of the gradient of the electrical potential is proportional to the

space density fo the electrical charge. This charge density is determined by the excess of the positive charge density over the negative charge density. Though at the inside far from boundary the fixed negative ion charge density is neutralized by the charge densities of the positive ions (potassium and sodium) there are in the inside, close to the surface, vacant negative sides. As has been pointed out before[21] these vacant sites, even if their concentration is too small to be determined chemically, play an essential role in the determination of the profile of the electrical potential. With the appropriate boundary conditions expressing that there is no force at large distances from the surface and electrical neutrality at those distances it is possible to find approximate analytical solutions of the (non-linear) differential equations for the electrical potential[6]. From these solutions it was found that the effective width of the electrical potential can be of the same order of magnitude as that of the membrane, as already mentioned above, indicating that the _functional_ membrane is an electrical phase boundary layer. Recognition of the inappropriateness of the assumption that there is no charge at the surface in a more realistic model, equations have been derived without that assumption. Proceeding as before[6] it is found that the equation which determines the electrical potential is a non-linear integral-differential equation which has to be solved with the appropriate boundary conditions. The solution of this non-linear integral-differential equation is, which of course a difficult problem, is being investigated[22]. It is not much more difficult to relax the assumption of the impermeability of the mobile anion into the inside phase[22]. As the fixed anionic sites in the inside phase are protein sites their interconnections have to be taken into account explicitly. This leads to the study of the changes in the distributions of electronic charges inside the sidechains and backbones of proteins. Such a study has to be made on the basis of wave-mechanics.

Wave-mechanical Treatment of Chemical Induction

Cooperative specific adsorption phenomena are based on the mechanism of chemical induction[23,24], which is the second aspect of the Association--Induction Hypothesis. This induction mechanism is a wave mechanical phenomenon. As the basis of wave mechanics is the Schrödinger equation, the latter has to be solved to understand the induction phenomena. Very unfortunately, the Schrödinger equation can only be exactly solved for very simple systems. For problems involving electrons in real atomic (and molecular) systems only approximate solutions have been obtained. Orginially, there were basically two different methods [25,26], the V(alence) B(ond) Method and the L(iner) C(ombination of) A(tomic) O(rbital) M(olecular) O(rbital) Method which are not only different from each other but also do not give the solution corresponding to the experimental data. There are several extensions of these methods[27], such as the self-consistent Hartree-Fock method, including configuration interaction and various degrees of overlap of atomic orbitals. The recently developed method[28] of Molecular Fragments which uses also Catastrophe Theory[29] holds great promise and is being investigated for application in this study. Though the separation is somewhat arbitrary, electrons in molecules have often been divided into two kinds: mobile electrons and localized electrons. The mobile electrons occur in molecules with double bonds, especially those in conjugated systems, over which they spread easily. However, for the induction phenomena involved in cooperative interactions, the localized electrons are the most important as they propagate electronic charge changes in a molecule from one atom to its neighbor. These localized electron charge changes have been studied by a theory especially developed for those localized electrons[30]. This theory has been applied to the study of the change in the electronic charges of linear polypeptides due to the desorption

of a calcium ion as a consequence of an applied cathodic field. Even if the
number of aminoacids is limited to the small number of five-for which, as
already stated above, the results of a stochastic treatment agree with those
on the basis of statistical mechanics (in which essentially an infinite
number of amino acids is assumed) - the number of equations which has to be
solved is large (in fact, seventy-five in the just-mentioned case of five
amino acids). The result of these computations is that the change in
electronic charge distribution due to the desorption of the cardinal
adsorbent calcium from the cardinal site does not propagate much further than
a few amino acids. The same result is obtained if this method is combined
with another method[31] using explicit transmission factors which decrease
with distance. This absence of sufficient far propagation of electronic
charge changes indicates that another important mechanism is also involved.
As in many fundamental biological processes conformational changes of
proteins occur probably also in physiological excitation[14]. These are due
to transformation of globularly extended peptide chains to helically coiled
peptide chains[19]. Such conformational changes of extended peptide chains,
which are interconnected by hydrogen bonded water molecules, can als be
studied theoretically by the above-mentioned methods[30,31] together with
other wave mechanical methods developed for the study of electrons in
biopolymers. The specific adsorption of water molecules at sites in
backbones of proteins have been studied taking into account their two and
three dimensional structures for which computer programs have been written.
When the results of the wave mechanical study will have been obtained they
will be used in these programs to obtain the multilayered polarized water
molecules adsorbed at the protein sites mentioned above.

Not only in physiological excitation but also in blood coagulation,
which is a beautiful example of regulatory phenomena in biology cooperative
phenomena play a very important role. Interesting results have recently been
obtained[32] for this cascade mechanism.

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